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E. Friedheim

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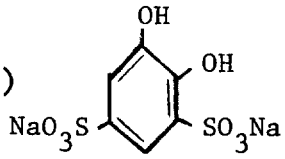
Dr. Joshua Lederberg, President
The Rockefeller University

Dear Joshua:

Thank you for the literature and your provocative suggestions on siderophores.

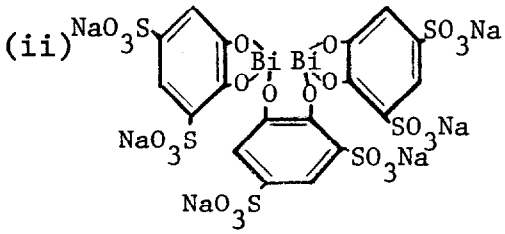
Your working hypothesis: siderophores carry Bi and As into the parasite - the metals compete with Fe for the siderophores and thereby deprive the parasite of Fe applies to the system Bi-phenolcatechols. These catechols chelate Bi and Fe but not As. I have prepared a water soluble Bi compound (ii) using a catechol chelator (i) well known for its affinity for Fe, i.e. 1,2-dihydroxybenzene-3,5-disulfonic acid.

(i)



tiron

(ii)



Bi_2tiron_3

As Bi is particularly active against spirochetes (Table 1), I have made arrangements with the following spirochete specialists to evaluate compound (ii) on spirochetes with a view to Lyme disease and periodontosis.

Dr. Sheena Waitkins, Director, Leptospira Reference Unit, Public Health Laboratory Service, County Hospital, Hereford, United Kingdom.

Dr. Walter Loesche, Professor of Dentistry, The University of Michigan School of Dentistry, Ann Arbor, Michigan.

Dr. Nils-Erik Fiehn, Department of Microbiology, Royal Dental College, Copenhagen, Denmark.

I consider this a first step and aim to prepare and evaluate other Bi chelates, in the first place, following your suggestion, with available siderophores. This amounts to a special project

requiring funding. Procter and Gamble are not interested because the primary causal role of spirochetes - and hence spirochetocidal agents - in periodontosis is not proved beyond doubt. Nevertheless, a number of experts, including my correspondents listed above, attribute to buccal spirochetes a significant contributory, if not primary, role in periodontosis.

It is of interest that the parasitocidal activity of the elements of column VA of the periodic table varies as shown:

Table 1

Parasitocidal activity of elements of column VA of periodic table

	Trypanosomes	Leishmania	Filariae	Spirochetes	Chelates Catechols
P	-	-	-	-	-
As	++	-	+	++	-
Sb	+	++	+	-	+
Bi	-	-	-	++	+
Hg	-	-	-	++	-

Of the parasites listed, only spirochetes are affected by Bi. Trypanosomes and filariae are sensitive to As and Sb, leishmania only to Sb. Hg, from another corner of the periodic table, is only active against spirochetes. The relation between atomic structure, chemical reactivity and biological effects remain to be unraveled. The pathway of parasitocidal metals (analogous to all material coming from outside of the cell to react with cell constituents) is (at least) two-tiered: a solubilizing "metalophore" transports the metal from the place of enteral or parenteral entry to a membrane receptor from where it interferes with a biochemical reaction.

In the case of the arsenicals, the first phase of the transport is assured by ligands, in my compounds represented by thiols. The identification of the As receptor is presently my main concern and is the subject matter of the enclosed recent grant application to N.I.H. To isolate As receptors, I am applying affinity chromatography with a parasitocidal arsenical as a target covalently bonded to sepharose. Parasite homogenates and fractions thereof are passed on columns charged with arsenated sepharose. In this work, I am ably assisted by Dr. M. Pflumm, formerly with Professor Edelman's laboratory at R.U. and for many years engaged at Columbia University with Professor

S. Beychok in protein chemistry, by Gabriel Ramirez, B.A., technician, and aided by the experience of Dr. H. Wood. I now have a compact unit with realistic prospects of making contributions to problems of metal transport and receptors along the lines you suggest, with a view to parasitocidal agents.

The crux is funding. The workhorse for the work with metals is atomic absorption or emission spectrometry. The Perkin-Elmer equipment which I salvaged from material discarded by Dr. Cerami has now come definitely to the end of its usefulness. Following your suggestion, I contacted Beckman Instruments to select the model best suited for my purposes. When I mentioned the name of Dr. Richard Nesbit, I was told that I could not count on a donation by Beckman. Could you possibly look into this critical situation?

I aim to apply the particular kind of chromatography with a chemotherapeutic molecule as a template outlined above, next to As, to Sb and Bi compounds. Here again, the problem is funding.

The part of your hypothesis relating parasitocidal Bi effects to the competition of Bi with Fe for siderophores, depriving the parasite of Fe, is a great challenge and I am eager to explore it, i.a. by the determination of the formation constants of the respective Bi and Fe complexes and by the possible compensatory effects of excess Fe supply.

A last thought pointing to a project of theoretical and practical significance: Nieland deplores the deficiencies in detecting siderophores, e.g. in spent media: "There has yet to be devised a single comprehensive, sensitive and reliable test for the siderophore ligand." I suggest that siderophores, i.e. chelates with a strong affinity to Fe(III), can be readily detected by the effect they are bound to have on the redox potential of a reference ferro-ferri system. For example, addition of a strong Fe(III) chelator, i.e. a siderophore, to a ferro-ferri citrate solution or tartrate solution would depress its redox potential. Applying the method of Michaelis and Friedheim (J. Biol. Chem., XCI: 343-353, 1931, enclosed), a large number of culture media could be rapidly screened for siderophores. This would require additional help and equipment. Cooperation with a laboratory engaged in this field would be welcome.

Most intriguing are the indications that binding sites for Fe and phage may coincide. This makes the search for the identity of these sites all the more desirable. Methods indicated above may help in this pursuit.

May I have your comment and advise regarding the above?

Yours sincerely,

Ernst